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(54) Title: **INDIVIDUALIZED ANTI-CANCER ANTIBODIES**

(57) Abstract: The present invention relates to a method for producing patient specific anti-cancer antibodies using a novel paradigm of screening. By segregating the anti-cancer antibodies using cancer cell cytotoxicity as end point, the process makes possible the production of anti-cancer antibodies customized for the individual patient that can be used for therapeutic and diagnostic purposes.

1 INDIVIDUALIZED ANTI-CANCER ANTIBODIES

2 **Reference to Related Applications:**

3 This application is a continuation-in-part of
4 application S.N. 09/415,278, filed October 8, 1999, now
5 U.S. Patent 6,180,357, the contents of which are herein
6 incorporated by reference.

7 **Field of the Invention:**

8 This invention relates to the production of anti-
9 cancer antibodies customized for the individual patient
10 which may be combined with chemotherapeutic agents that
11 can be used for therapeutic and diagnostic purposes. The
12 invention further relates to the process by which the
13 antibodies are made and to their methods of use.

14

15 **Background of the Invention:**

16 Each individual who presents with cancer is unique
17 and has a cancer that is as different from other cancers
18 as that person's identity. Despite this, current therapy
19 treats all patients with the same type of cancer, at the
20 same stage, in the same way. At least 30% of these
21 patients will fail the first line therapy, thus leading to
22 further rounds of treatment and the increased probability
23 of treatment failure, metastases, and ultimately, death.
24 A superior approach to treatment would be the
25 customization of therapy for the particular individual.
26 The only current therapy which lends itself to
27 customization is surgery. Chemotherapy and radiation
28 treatment can not be tailored to the patient, and surgery
29 by itself, in most cases is inadequate for producing
30 cures.

31 With the advent of monoclonal antibodies, the
32 possibility of developing methods for customized therapy
33 became more realistic since each antibody can be directed
34 to a single epitope. Furthermore, it is possible to
35 produce a combination of antibodies that are directed to

1 the constellation of epitopes that uniquely define a
2 particular individual's tumor.

3 Having recognized that a significant difference
4 between cancerous and normal cells is that cancerous cells
5 contain antigens that are specific to transformed cells,
6 the scientific community has long held that monoclonal
7 antibodies can be designed to specifically target
8 transformed cells by binding specifically to these cancer
9 antigens; thus giving rise to the belief that monoclonal
10 antibodies can serve as "Magic Bullets" to eliminate
11 cancer cells.

12 At the present time, however, the cancer patient
13 usually has few options of treatment. The regimented
14 approach to cancer therapy has produced improvements in
15 global survival and morbidity rates. However, to the
16 particular individual, these improved statistics do not
17 necessarily correlate with an improvement in their
18 personal situation.

19 Thus, if a methodology was put forth which enabled
20 the practitioner to treat each tumor independently of
21 other patients in the same cohort, this would permit the
22 unique approach of tailoring therapy to just that one
23 person. Such a course of therapy would, ideally, increase
24 the rate of cures, and produce better outcomes, thereby
25 satisfying a long-felt need.

26 Historically, the use of polyclonal antibodies has
27 been used with limited success in the treatment of human
28 cancers. Lymphomas and leukemias have been treated with
29 human plasma, but there were few prolonged remission or
30 responses. Furthermore, there was a lack of
31 reproducibility and there was no additional benefit
32 compared to chemotherapy. Solid tumors such as breast
33 cancers, melanomas and renal cell carcinomas have also
34 been treated with human blood, chimpanzee serum, human

1 plasma and horse serum with correspondingly unpredictable
2 and ineffective results.

3 There have been many clinical trials of monoclonal
4 antibodies for solid tumors. In the 1980s there were at
5 least four clinical trials for human breast cancer which
6 produced only one responder from at least 47 patients
7 using antibodies against specific antigens or based on
8 tissue selectivity. It was not until 1998 that there was
9 a successful clinical trial using a humanized anti-her 2
10 antibody in combination with Cisplatin. In this trial 37
11 patients were accessed for responses of which about a
12 quarter had a partial response rate and another half had
13 minor or stable disease progression.

14 The clinical trials investigating colorectal cancer
15 involve antibodies against both glycoprotein and
16 glycolipid targets. Antibodies such as 17-1A, which has
17 some specificity for adenocarcinomas, had undergone Phase
18 2 clinical trials in over 60 patients with only one
19 patient having a partial response. In other trials, use
20 of 17-1A produced only one complete response and two minor
21 responses among 52 patients in protocols using additional
22 cyclophosphamide. Other trials involving 17-1A yielded
23 results that were similar. The use of a humanized murine
24 monoclonal antibody initially approved for imaging also
25 did not produce tumor regression. To date there has not
26 been an antibody that has been effective for colorectal
27 cancer. Likewise there have been equally poor results for
28 lung cancer, brain cancers, ovarian cancers, pancreatic
29 cancer, prostate cancer, and stomach cancer. There has
30 been some limited success in the use of anti-GD3
31 monoclonal antibody for melanoma. Thus, it can be seen
32 that despite successful small animal studies that are a
33 prerequisite for human clinical trials, the antibodies
34 that have been tested have been for the most part
35 ineffective.

1 **Prior Patents:**

2 U.S. Patent No. 5,750,102 discloses a process wherein
3 cells from a patient's tumor are transfected with MHC
4 genes which may be cloned from cells or tissue from the
5 patient. These transfected cells are then used to
6 vaccinate the patient.

7 U.S. Patent No. 4,861,581 discloses a process
8 comprising the steps of obtaining monoclonal antibodies
9 that are specific to an internal cellular component of
10 neoplastic and normal cells of the mammal but not to
11 external components, labeling the monoclonal antibody,
12 contacting the labeled antibody with tissue of a mammal
13 that has received therapy to kill neoplastic cells, and
14 determining the effectiveness of therapy by measuring the
15 binding of the labeled antibody to the internal cellular
16 component of the degenerating neoplastic cells. In
17 preparing antibodies directed to human intracellular
18 antigens, the patentee recognizes that malignant cells
19 represent a convenient source of such antigens.

20 U.S. Patent No. 5,171,665 provides a novel antibody
21 and method for its production. Specifically, the patent
22 teaches formation of a monoclonal antibody which has the
23 property of binding strongly to a protein antigen
24 associated with human tumors, e.g. those of the colon and
25 lung, while binding to normal cells to a much lesser
26 degree.

27 U.S. Patent No. 5,484,596 provides a method of cancer
28 therapy comprising surgically removing tumor tissue from a
29 human cancer patient, treating the tumor tissue to obtain
30 tumor cells, irradiating the tumor cells to be viable but
31 non-tumorigenic, and using these cells to prepare a
32 vaccine for the patient capable of inhibiting recurrence
33 of the primary tumor while simultaneously inhibiting
34 metastases. The patent teaches the development of
35 monoclonal antibodies which are reactive with surface
36 antigens of tumor cells. As set forth at col. 4, lines 45

1 et seq., the patentees utilize autochthonous tumor cells
2 in the development of monoclonal antibodies expressing
3 active specific immunotherapy in human neoplasia.

4 U.S. Patent No. 5,693,763 teaches a glycoprotein
5 antigen characteristic of human carcinomas and not
6 dependent upon the epithelial tissue of origin.

7 U.S. Patent No. 5,783,186 is drawn to Anti-Her2
8 antibodies which induce apoptosis in Her2 expressing
9 cells, hybridoma cell lines producing the antibodies,
10 methods of treating cancer using the antibodies and
11 pharmaceutical compositions including said antibodies.

12 U.S. Patent No. 5,849,876 describes new hybridoma
13 cell lines for the production of monoclonal antibodies to
14 mucin antigens purified from tumor and non-tumor tissue
15 sources.

16 U.S. Patent No. 5,869,268 is drawn to a method for
17 producing a human lymphocyte producing an antibody
18 specific to a desired antigen, a method for producing a
19 monoclonal antibody, as well as monoclonal antibodies
20 produced by the method. The patent is particularly drawn
21 to the production of an anti-HD human monoclonal antibody
22 useful for the diagnosis and treatment of cancers.

23 U.S. Patent No. 5,869,045 relates to antibodies,
24 antibody fragments, antibody conjugates and single chain
25 immunotoxins reactive with human carcinoma cells. The
26 mechanism by which these antibodies function is two-fold,
27 in that the molecules are reactive with cell membrane
28 antigens present on the surface of human carcinomas, and
29 further in that the antibodies have the ability to
30 internalize within the carcinoma cells, subsequent to
31 binding, making them especially useful for forming
32 antibody-drug and antibody-toxin conjugates. In their
33 unmodified form the antibodies also manifest cytotoxic
34 properties at specific concentrations.

35 U.S. Patent No. 5,780,033 discloses the use of
36 autoantibodies for tumor therapy and prophylaxis. However,

1 this antibody is an antinuclear autoantibody from an aged
2 mammal. In this case, the autoantibody is said to be one
3 type of natural antibody found in the immune system.
4 Because the autoantibody comes from "an aged mammal",
5 there is no requirement that the autoantibody actually
6 comes from the patient being treated. In addition the
7 patent discloses natural and monoclonal antinuclear
8 autoantibody from an aged mammal, and a hybridoma cell
9 line producing a monoclonal antinuclear autoantibody.

10

11 **Summary of the Invention:**

12 This application teaches a method for producing
13 patient specific anti-cancer antibodies using a novel
14 paradigm of screening. These antibodies can be made
15 specifically for one tumor and thus make possible the
16 customization of cancer therapy. Within the context of
17 this application, anti-cancer antibodies having either
18 cell-killing (cytotoxic) or cell-growth inhibiting
19 (cytostatic) properties will hereafter be referred to as
20 cytotoxic. These antibodies can be used in aid of staging
21 and diagnosis of a cancer, and can be used to treat tumor
22 metastases.

23 The prospect of individualized anti-cancer treatment
24 will bring about a change in the way a patient is managed.
25 A likely clinical scenario is that a tumor sample is
26 obtained at the time of presentation, and banked. From
27 this sample, the tumor can be typed from a panel of pre-
28 existing anti-cancer antibodies. The patient will be
29 conventionally staged but the available antibodies can be
30 of use in further staging the patient. The patient can be
31 treated immediately with the existing antibodies, and a
32 panel of antibodies specific to the tumor can be produced
33 either using the methods outlined herein or through the
34 use of phage display libraries in conjunction with the
35 screening methods herein disclosed. All the antibodies
36 generated will be added to the library of anti-cancer

1 antibodies since there is a possibility that other tumors
2 can bear some of the same epitopes as the one that is
3 being treated.

4 In addition to anti-cancer antibodies, the patient
5 can elect to receive the currently recommended therapies
6 as part of a multi-modal regimen of treatment. The fact
7 that the antibodies isolated via the present methodology
8 are relatively non-toxic to non-cancerous cells allow
9 combinations of antibodies at high doses to be used,
10 either alone, or in conjunction with conventional therapy.
11 The high therapeutic index will also permit re-treatment
12 on a short time scale that should decrease the likelihood
13 of emergence of treatment resistant cells.

14 If the patient is refractory to the initial course of
15 therapy or metastases develop, the process of generating
16 specific antibodies to the tumor can be repeated for re-
17 treatment. Furthermore, the anti-cancer antibodies can be
18 conjugated to red blood cells obtained from that patient
19 and re-infused for treatment of metastases. There have
20 been few effective treatments for metastatic cancer and
21 metastases usually portend a poor outcome resulting in
22 death. However, metastatic cancers are usually well
23 vascularized and the delivery of anti-cancer antibodies by
24 red blood cells can have the effect of concentrating the
25 antibodies at the site of the tumor. Even prior to
26 metastases, most cancer cells are dependent on the host's
27 blood supply for their survival and anti-cancer antibody
28 conjugated red blood cells can be effective against *in*
29 *situ* tumors, too. Alternatively, the antibodies may be
30 conjugated to other hematogenous cells, e.g. lymphocytes,
31 macrophages, monocytes, natural killer cells, etc.

32

33 There are five classes of antibodies and each is
34 associated with a function that is conferred by its heavy
35 chain. It is generally thought that cancer cell killing

1 by naked antibodies are mediated either through antibody
2 dependent cellular cytotoxicity or complement dependent
3 cytotoxicity. For example murine IgM and IgG2a antibodies
4 can activate human complement by binding the C-1 component
5 of the complement system thereby activating the classical
6 pathway of complement activation which can lead to tumor
7 lysis. For human antibodies the most effective complement
8 activating antibodies are generally IgM and IgG1. Murine
9 antibodies of the IgG2a and IgG3 isotype are effective at
10 recruiting cytotoxic cells that have Fc receptors which
11 will lead to cell killing by monocytes, macrophages,
12 granulocytes and certain lymphocytes. Human antibodies of
13 both the IgG1 and IgG3 isotype mediate ADCC.

14 Another possible mechanism of antibody mediated
15 cancer killing may be through the use of antibodies that
16 function to catalyze the hydrolysis of various chemical
17 bonds in the cell membrane and its associated
18 glycoproteins or glycolipids, so-called catalytic
19 antibodies.

20 There are two additional mechanisms of antibody
21 mediated cancer cell killing which are more widely
22 accepted. The first is the use of antibodies as a vaccine
23 to induce the body to produce an immune response against
24 the putative cancer antigen that resides on the tumor
25 cell. The second is the use of antibodies to target
26 growth receptors and interfere with their function or to
27 down regulate that receptor so that effectively its
28 function is lost.

29 Accordingly, it is an objective of the invention to
30 teach a method for producing anti-cancer antibodies from
31 cells derived from a particular individual which are
32 cytotoxic with respect to cancer cells while
33 simultaneously being relatively non-toxic to non-cancerous
34 cells.

35 It is an additional objective of the invention to
36 produce novel anti-cancer antibodies.

1 It is a further objective of the instant invention to
2 produce anti-cancer antibodies whose cytotoxicity is
3 mediated through antibody dependent cellular toxicity.

4 It is yet an additional objective of the instant
5 invention to produce anti-cancer antibodies whose
6 cytotoxicity is mediated through complement dependent
7 cellular toxicity.

8 It is still a further objective of the instant
9 invention to produce anti-cancer antibodies whose
10 cytotoxicity is a function of their ability to catalyze
11 hydrolysis of cellular chemical bonds.

12 Still an additional objective of the instant
13 invention is to produce anti-cancer antibodies useful as a
14 vaccine to produce an immune response against putative
15 cancer antigen residing on tumor cells.

16 A further objective of the instant invention is the
17 use of antibodies to target cell membrane proteins, such
18 as growth receptors, cell membrane pumps and cell
19 anchoring proteins, thereby interfering with or down
20 regulating their function.

21 Yet an additional objective of the instant invention
22 is the production of anti-cancer antibodies whose cell-
23 killing utility is concomitant with their ability to
24 effect a conformational change in cellular proteins such
25 that a signal will be transduced to initiate cell-killing.

26 A still further objective of the instant invention is
27 to produce anti-cancer antibodies which are useful for
28 diagnosis, prognosis, and monitoring of cancer, e.g.
29 production of a panel of therapeutic anti-cancer
30 antibodies to test patient samples to determine if they
31 contain any suitable antibodies for therapeutic use.

32 Yet another objective of the instant invention is to
33 produce novel antigens, associated with cancer processes,
34 which can be discovered by using anti-cancer antibodies
35 derived by the process of the instant invention. These

1 antigens are not limited to proteins, as is generally the
2 case with genomic data; they may also be derived from
3 carbohydrates or lipids or combinations thereof.

4 Other objects and advantages of this invention will
5 become apparent from the following description wherein are
6 set forth, by way of illustration and example, certain
7 embodiments of this invention.

8

9 **Detailed Description of the Invention:**

10 It is to be understood that while a certain form of
11 the invention is illustrated, it is not to be limited to
12 the specific form or arrangement herein described and
13 shown. It will be apparent to those skilled in the art
14 that various changes may be made without departing from
15 the scope of the invention and the invention is not to be
16 considered limited to what is shown and described in the
17 specification.

18 One of the potential benefits of monoclonal
19 antibodies with respect to the treatment of cancer is
20 their ability to specifically recognize single antigens.
21 It was thought that in some instances cancer cells possess
22 antigens that were specific to that kind of transformed
23 cell. It is now more frequently believed that cancer
24 cells have few unique antigens, rather, they tend to over-
25 express a normal antigen or express fetal antigens.
26 Nevertheless, the use of monoclonal antibodies provided a
27 method of delivering reproducible doses of antibodies to
28 the patient with the expectation of better response rates
29 than with polyclonal antibodies.

30 Traditionally, monoclonal antibodies have been made
31 according to fundamental principles laid down by Kohler
32 and Milstein. Mice are immunized with antigens, with or
33 without, adjuvants. The splenocytes are harvested from
34 the spleen for fusion with immortalized hybridoma
35 partners. These are seeded into microtitre plates where

1 they can secrete antibodies into the supernatant that is
2 used for cell culture. To select from the hybridomas that
3 have been plated for the ones that produce antibodies of
4 interest the hybridoma supernatants are usually tested for
5 antibody binding to antigens in an ELISA (enzyme linked
6 immunosorbent assay) assay. The idea is that the wells
7 that contain the hybridoma of interest will contain
8 antibodies that will bind most avidly to the test antigen,
9 usually the immunizing antigen. These wells are then
10 subcloned in limiting dilution fashion to produce
11 monoclonal hybridomas. The selection for the clones of
12 interest is repeated using an ELISA assay to test for
13 antibody binding. Therefore, the principle that has been
14 propagated is that in the production of monoclonal
15 antibodies the hybridomas that produce the most avidly
16 binding antibodies are the ones that are selected from
17 among all the hybridomas that were initially produced.
18 That is to say, the preferred antibody is the one with
19 highest affinity for the antigen of interest.

20 There have been many modifications of this procedure
21 such as using whole cells for immunization. In this
22 method, instead of using purified antigens, entire cells
23 are used for immunization. Another modification is the
24 use of cellular ELISA for screening. In this method
25 instead of using purified antigens as the target in the
26 ELISA, fixed cells are used. In addition to ELISA tests,
27 complement mediated cytotoxicity assays have also been
28 used in the screening process. However, antibody-binding
29 assays were used in conjunction with cytotoxicity tests.
30 Thus, despite many modifications, the process of producing
31 monoclonal antibodies relies on antibody binding to the
32 test antigen as an endpoint.

33 Most antibodies directed against cancer cells have
34 been produced using the traditional methods outlined
35 above. These antibodies have been used both
36 therapeutically and diagnostically. In general, for both

1 these applications, the antibody has been used as the
2 targeting agent that delivers a payload to the site of the
3 cancer. These antibody conjugates can either be
4 radioactive, toxic, or serve as an intermediary for
5 further delivery of a drug to the body, such as an enzyme
6 or biotin. Furthermore, it was widely held, until
7 recently, that naked antibodies had little effect *in vivo*.
8 Both HERCEPTIN and RITUXIMAB are humanized murine
9 monoclonal antibodies that have recently been approved for
10 human use by the FDA. However, both these antibodies were
11 initially made by assaying for antibody binding and their
12 direct cytotoxicity was not the primary goal during the
13 production of hybridomas. Any tendency for these
14 antibodies to produce tumor cell killing is thus through
15 chance, not by design.

16 Although the production of monoclonal antibodies have
17 been carried out using whole cell immunization for various
18 applications the screening of these hybridomas have relied
19 on either putative or identified target antigens or on the
20 selectivity of these hybridomas for specific tissues. It
21 is axiomatic that the best antibodies are the ones with
22 the highest binding constants. This concept originated
23 from the basic biochemical principle that enzymes with the
24 highest binding constants were the ones that were the most
25 effective for catalyzing a reaction. This concept is
26 applicable to receptor ligand binding where the drug
27 molecule binding to the receptor with the greatest
28 affinity usually has the highest probability for
29 initiating or inhibiting a signal. However, this may not
30 always be the case since it is possible that in certain
31 situations there may be cases where the initiation or
32 inhibition of a signal may be mediated through non-
33 receptor binding. The information conveyed by a
34 conformational change induced by ligand binding can have
35 many consequences such as a signal transduction,
36 endocytosis, among the others. The ability to produce a

1 conformational change in a receptor molecule may not
2 necessarily be due to the filling of a ligand receptor
3 pocket but may occur through the binding of another extra
4 cellular domain or due to receptor clustering induced by a
5 multivalent ligand.

6 The production of antibodies to produce cell killing
7 need not be predicated upon screening of the hybridomas
8 for the best binding antibodies. Rather, although not
9 advocated by those who produce monoclonal antibodies, the
10 screening of the hybridoma supernatants for cell killing
11 or alternatively for cessation of growth of the cancerous
12 cells may be selected as a desirable endpoint for the
13 production of cytotoxic or cytostatic antibodies. It is
14 well understood that the *in-vivo* antibodies mediate their
15 function through the Fc portions and that the utility of
16 the therapeutic antibody is determined by the
17 functionality of the constant region or attached moieties.
18 In this case the FAB portion of the antibody, the antigen-
19 combining portion, will confer to the antibody its
20 specificity and the Fc portion its functionality. The
21 antigen combining site of the antibody can be considered
22 to be the product of a natural combinatorial library. The
23 result of the rearrangement of the variable region of the
24 antibody can be considered a molecular combinatorial
25 library where the output is a peptide. Therefore, the
26 sampling of this combinatorial library can be based on any
27 parameter. Like sampling a natural compound library for
28 antibiotics, it is possible to sample an antibody library
29 for cytotoxic or cytostatic compounds.

30 The various endpoints in a screen must be
31 differentiated from each other. For example, the
32 difference between antibody binding to the cell is
33 distinct from cell killing. Cell killing (cytotoxicity) is
34 distinct from the mechanisms of cell death such as oncosis
35 or apoptosis. There can be many processes by which cell
36 death is achieved and some of these can lead either to

1 oncosis or apoptosis. There is speculation that there are
2 other cell death mechanisms other than oncosis or
3 apoptosis but regardless of how the cell arrives at death
4 there are some commonalities of cell death. One of these
5 is the absence of metabolism and another is the
6 denaturation of enzymes. In either case vital stains will
7 fail to stain these cells. These endpoints of cell death
8 have been long understood and predate the current
9 understanding of the mechanisms of cell death.
10 Furthermore, there is the distinction between cytotoxic
11 effects where cells are killed and cytostatic effects
12 where the proliferation of cells are inhibited.

13 In a preferred embodiment of the present invention,
14 the assay is conducted by focusing on cytotoxic activity
15 toward cancerous cells as an end point. In a preferred
16 embodiment, a live /dead assay kit , for example the
17 LIVE/DEAD® Viability/Cytotoxicity Assay Kit (L-3224) by
18 Molecular Probes, is utilized. The Molecular Probes kit
19 provides a two-color fluorescence cell viability assay
20 that is based on the simultaneous determination of live
21 and dead cells with two probes that measure two recognized
22 parameters of cell viability - intracellular esterase
23 activity and plasma membrane integrity. The assay
24 principles are general and applicable to most eukaryotic
25 cell types, including adherent cells and certain tissues,
26 but not to bacteria or yeast. This fluorescence-based
27 method of assessing cell viability is preferred in place
28 of such assays as trypan blue exclusion, Cr release and
29 similar methods for determining cell viability and
30 cytotoxicity.

31 In carrying out the assay, live cells are
32 distinguished by the presence of ubiquitous intracellular
33 esterase activity, determined by the enzymatic conversion
34 of the virtually nonfluorescent cell-permeant CALCEIN AM
35 to the intensely fluorescent Calcein. The polyanionic dye
36 Calcein is well retained within live cells, producing an

1 intense uniform green fluorescence in live cells (ex/em
2 ~495 nm/~515 nm). EthD-1 enters cells with damaged
3 membranes and undergoes a 40-fold enhancement of
4 fluorescence upon binding to nucleic acids, thereby
5 producing a bright red fluorescence in dead cells (ex/em
6 ~495 nm/~635 nm). EthD-1 is excluded by the intact plasma
7 membrane of live cells. The determination of cell
8 viability depends on these physical and biochemical
9 properties of cells. Cytotoxic events that do not affect
10 these cell properties may not be accurately assessed using
11 this method. Background fluorescence levels are inherently
12 low with this assay technique because the dyes are
13 virtually nonfluorescent before interacting with cells.

14 In addition to the various endpoints for screening,
15 there are two other major characteristics of the screening
16 process. The library of antibody gene products is not a
17 random library but is the product of a biasing procedure.
18 In the examples below, the biasing is produced by
19 immunizing mice with fixed cells. This increases the
20 proportion of antibodies that have the potential to bind
21 the target antigen. Although immunization is thought of as
22 a way to produce higher affinity antibodies (affinity
23 maturation) in this case it is not. Rather, it can be
24 considered as a way to shift the set of antigen combining
25 sites towards the targets. This is also distinct from the
26 concept of isotype switching where the functionality, as
27 dictated by the constant portion of the heavy chain, is
28 altered from the initial IgM isotype to another isotype
29 such as IgG.

30 The third key feature that is crucial in the
31 screening process is the use of multitarget screening. To
32 a certain extent specificity is related to affinity. An
33 example of this is the situation where an antigen has very
34 limited tissue distribution and the affinity of the
35 antibody is a key determinant of the specificity of the

1 antibody-the higher the affinity the more tissue specific
2 the antibody and likewise an antibody with low affinity
3 may bind to tissues other than the one of interest.
4 Therefore, to address the specificity issue the antibodies
5 are screened simultaneously against a variety of cells. In
6 the examples below the hybridoma supernatants
7 (representing the earliest stages of monoclonal antibody
8 development), are tested against a number of cell lines to
9 establish specificity as well as activity.

10 The antibodies are designed for therapeutic treatment
11 of cancer in patients. Ideally the antibodies can be naked
12 antibodies. They can also be conjugated to toxins. They
13 can be used to target other molecules to the cancer. e.g.
14 biotin conjugated enzymes. Radioactive compounds can also
15 be used for conjugation.

16 The antibodies can be fragmented and rearranged
17 molecularly. For example Fv fragments can be made; sFv-
18 single chain Fv fragments; diabodies etc.

19 It is envisioned that these antibodies can be used
20 for diagnosis, prognosis, and monitoring of cancer. For
21 example the patients can have blood samples drawn for shed
22 tumor antigens which can be detected by these antibodies
23 in different formats such as ELISA assays, rapid test
24 panel formats etc. The antibodies can be used to stain
25 tumor biopsies for the purposes of diagnosis. In addition
26 a panel of therapeutic antibodies can be used to test
27 patient samples to determine if there are any suitable
28 antibodies for therapeutic use.

29 **Example one**

30 In order to produce monoclonal antibodies specific
31 for a tumor sample the method of selection of the
32 appropriate hybridoma wells is complicated by the
33 probability of selecting wells which will produce false
34 positive signals. That is to say that there is the

1 likelihood of producing antibodies that can react against
2 normal cells as well as cancer cells. To obviate this
3 possibility one strategy is to mask the anti-normal
4 antigen antibodies from the selection process. This can
5 be accomplished by removing the anti-normal antibodies at
6 the first stage of screening thereby revealing the
7 presence of the desired antibodies. Subsequent limiting
8 dilution cloning can delineate the clones that will not
9 produce killing of control cells but will produce target
10 cancer cell killing.

11 Biopsy specimens of breast, melanoma, and lung tumors
12 were obtained and stored at -70°C until used. Single cell
13 suspensions were prepared and fixed with -30°C , 70%
14 ethanol, washed with PBS and reconstituted to an
15 appropriate volume for injection. Balb/c mice were
16 immunized with 2.5×10^5 - 1×10^6 cells and boosted every third
17 week until a final pre-fusion boost was performed three
18 days prior to the splenectomy. The hybridomas were
19 prepared by fusing the isolated splenocytes with Sp2/0 and
20 NS1 myeloma partners. The supernatants from the fusions
21 were tested for subcloning of the hybridomas.

22 Cells (including A2058 melanoma cells, CCD-12CoN
23 fibroblasts, MCF-12A breast cells among others) were
24 obtained from ATCC and cultured according to enclosed
25 instructions. The HEY cell line was a gift from Dr. Inka
26 Brockhausen. The non-cancer cells, e.g. CCD-12CoN
27 fibroblasts and MCF-12A breast cells, were plated into 96-
28 well microtitre plates (NUNC) 1 to 2 weeks prior to
29 screening. The cancer cells, e.g. HEY, A2058, BT 483, and
30 HS294t, were plated two or three days prior to screening.

31 The plated normal cells were fixed prior to use. The
32 plates were washed with 100 microliters of PBS for 10
33 minutes at room temperature and then aspirated dry. 75
34 microliters of 0.01 percent glutaraldehyde diluted in PBS
35 were added to each well for five minutes and then
36 aspirated. The plates were washed with 100 microliters of

1 PBS three times at room temperature. The wells were
2 emptied and 100 microliters of one percent human serum
3 albumin in PBS was added to each well for one hour at room
4 temperature. The plates were then stored at four degrees
5 Celsius.

6 Prior to the transfer of the supernatant from the
7 hybridoma plates the fixed normal cells were washed three
8 times with 100 microliters of PBS at room temperature.
9 After aspiration to the microliters of the primary
10 hybridoma culture supernatants were transferred to the
11 fixed cell plates and incubated for two hours at 37
12 degrees Celsius in a 8 percent CO₂ incubator. The
13 hybridoma supernatants derived from melanoma was incubated
14 with CCD-12 CoN cells and those derived from breast cancer
15 were incubated with MCF-12a cells. After incubation
16 the absorbed supernatant was divided into two 75
17 microliter portions and transferred to target cancer cell
18 plates. Prior to the transfer the cancer cell plates were
19 washed three times with 100 microliters of PBS. The
20 supernatant from the CCD-12 CoN cells were transferred to
21 the A2058 and the HS294t cells, whereas the supernatant
22 from MCF-12A cells were transferred to the HEY and BT 483
23 cells. The cancer cells were incubated with the hybridoma
24 supernatants for 18 hours at 37 degrees Celsius in an 8
25 percent CO₂ incubator.

26 The Live/Dead cytotoxicity assay was obtained from
27 Molecular Probes (Eu,OR). The assays were performed
28 according to the manufacturer's instructions with the
29 changes outlined below. The plates with the cells were
30 washed once with 100 microliters of PBS at 37°C. 75 to 100
31 microliters of supernatant from the hybridoma microtitre
32 plates were transferred to the cell plates and incubated
33 in a 8% CO₂ incubator for 18-24 hours. Then, the wells that
34 served as the all dead control were aspirated until empty
35 and 50 microliters of 70% ethanol was added. The plate was
36 then emptied by inverting and blotted dry. Room

1 temperature PBS was dispensed into each well from a
2 multichannel squeeze bottle, tapped three times, emptied
3 by inversion and then blotted dry. 50 microliters of the
4 fluorescent Live/Dead dye diluted in PBS was added to each
5 well and incubated at 37°C in a 5% CO₂ incubator for one
6 hour. The plates were read in a Perkin-Elmer HTS7000
7 fluorescence plate reader and the data was analyzed in
8 Microsoft Excel.

9 Four rounds of screening were conducted to produce
10 single clone hybridoma cultures. For two rounds of
11 screening the hybridoma supernatants were tested only
12 against the cancer cells. In the last round of screening
13 the supernatant was tested against a number of non-cancer
14 cells as well as the target cells indicated in table 1.
15 The antibodies were isotyped using a commercial isotyping
16 kit.

17 A number of monoclonal antibodies were produced in
18 accordance with the method of the present invention.
19 These antibodies, whose characteristics are summarized in
20 Table 1, are identified as 3BD-3, 3BD-6, 3BD-8, 3BD-9,
21 3BD-15, 3BD-25, 3BD-26 and 3BD-27. Each of the designated
22 antibodies is produced by a hybridoma cell line deposited
23 with the American Type Culture Collection at 10801
24 University Boulevard, Manassas, Va. having an ATCC
25 Accession Number as follows:

26	<u>Antibody</u>	<u>ATCC Accession Number</u>
27	3BD-3	
28	3BD-6	
29	3BD-8	
30	3BD-9	
31	3BD-15	
32	3BD-25	
33	3BD-26	
34	3BD-27	

1 These antibodies are considered monoclonal after four
 2 rounds of limiting dilution cloning. The anti-melanoma
 3 antibodies did not produce significant cancer cell
 4 killing. The panel of anti-breast cancer antibodies killed
 5 32-87% of the target cells and <1-3% of the control cells.
 6 The predominant isotype was IgG1 even though it was
 7 expected that the majority of anti-tumor antibodies would
 8 be directed against carbohydrate antigens, and thus, be of
 9 the IgM type. There is a high therapeutic index since most
 10 antibodies spare the control cells from cell death.

11 Table 1. Anti-Breast Cancer Antibodies

12

13

14

Clones	% Cell Death			
	Target for Anti-Breast Cancer Antibodies (HEY & A2058)	Normal Fibroblast Cells (CCD-12CoN)	Fibrocystic Breast Cells (MCF-12A)	Isotype
15 3BD-3	74.9%	3.7%	<1%	$\gamma 1, \lambda$
16 3BD-6	68.5%	5.6%	<1%	$\gamma 1, \lambda$
17 3BD-8	81.9%	4.5%	2.6%	$\gamma 1, \kappa$
18 3BD-9	77.2%	7.9%	<1%	$\gamma 1, \lambda$
19 3BD-15	87.1%	<1 %	<1%	$\gamma 1, \lambda$
20 3BD-26	54.8%	3.3%	<1%	μ, κ
21 3BD-25	32.4%	3.6%	<1 %	$\gamma 1, \kappa$
22 3BD-27	60.1%	8.3%	1.3%	$\gamma 1, \kappa$

23

24 **Example 2**

25 In this example customized anti-cancer antibodies are
 26 produced by first obtaining samples of the patient's
 27 tumor. Usually this is from a biopsy specimen from a
 28 solid tumor or a blood sample from hematogenous tumors.
 29 The samples are prepared into single cell suspensions and
 30 fixed for injection into mice. After the completion of the
 31 immunization schedule the hybridomas are produced from the
 32 splenocytes. The hybridomas are screened against a variety

1 of cancer cell lines and normal cells in standard
2 cytotoxicity assays. Those hybridomas that are reactive
3 against cancer cell lines but are not reactive against
4 normal non-transformed cells are selected for further
5 propagation. Clones that were considered positive were
6 ones that selectively killed the cancer cells but did not
7 kill the non-transformed cells. The antibodies are
8 characterized for a large number of biochemical parameters
9 and then humanized for therapeutic use.

10 The melanoma tumor cells isolated and cell lines were
11 cultured as described in Example 1. Balb/c mice were
12 immunized according to the following schedule: 200,000
13 cells s.c. and i.p. on day 0, then 200,000 cells were
14 injected i.p. on day 21, then 1,000,000 cells were
15 injected on day 49, then 1,250,000 cells in Freund's
16 Complete Adjuvant were injected i.p. on day 107, and then
17 200,000 cells were injected on day 120 i.p. and then the
18 mice were sacrificed on day 123. The spleens were
19 harvested and the splenocytes were divided into two
20 aliquots for fusion with Sp2/0 (1LN) or NS-1 (2LN) myeloma
21 partners using the methods outlined in example 1.

22 The screening was carried out 11 days after the
23 fusion against A2058 melanoma cells and CCD-12CoN
24 fibroblasts. Each pair of plates were washed with 100
25 microliters of room temperature PBS and then aspirated to
26 near dryness. Then 50 microliters of hybridoma supernatant
27 was added to the same wells on each of the two plates. The
28 spent Sp2/0 supernatant was added to the control wells at
29 the same volume and the plates were incubated for around
30 18 hours at 37 degrees Celsius at a 8%CO₂, 98% relative
31 humidity incubator. Then each pair of plates were removed
32 and in the positive control wells 50 microliters of 70%
33 ethanol was substituted for the media for 4 seconds. The
34 plates were then inverted and washed with room temperature
35 PBS once and dried. Then 50uL of fluorescent live/dead dye
36 diluted in PBS (Molecular Probes Live/Dead Kit) was added

1 for one hour and incubated at 37 degrees Celsius. The
2 plates were then read in a Perkin-Elmer fluorescent plate
3 reader and the data analyzed using Microsoft Excel. The
4 wells that were considered positive were subcloned and the
5 same screening process was repeated 13 days later and then
6 33 days later. The results of the last screening is
7 outlined in Table 2 below. A number of monoclonal
8 antibodies were produced in accordance with the method of
9 the present invention. These antibodies, whose
10 characteristics are summarized in Table 2, are identified
11 as 1LN-1, 1LN-8, 1LN-12, 1LN-14, 2LN-21, 2LN-28, 2LN-29,
12 2LN-31, 2LN-33, 2LN-34 and 2LN-35. Each of the designated
13 antibodies is produced by a hybridoma cell line deposited
14 with the American Type Culture Collection at 10801
15 University Boulevard, Manassas, Va. having an ATCC
16 Accession Number as follows:

17

18

19	<u>Antibody</u>	<u>ATCC Accession Number</u>
20	1LN-1	
21	1LN-8	
22	1LN-12	
23	1LN-14	
24	2LN-21	
25	2LN-28	
26	2LN-29	
27	2LN-31	
28	2LN-33	
29	2LN-34	
30	2LN-35	

Table 2, Anti-Melanoma Antibodies

Clones	% Cell Death	
	Target for Anti-Melanoma Antibodies (A2058)	Normal Fibroblast Cells (CCD-1 2CoN)
1LN-1	59.4%	<1 %
1LN-8	11.0%	5.0%
1LN-12	55.2%	1.4%
1LN-14	51.4%	<1%
2LN-21	72.0%	15.9%
2LN-28	66.6%	12.4%
2LN-29	78.2%	6.1%
2LN-31	100%	7.8%
2LN-33	94.2%	<1%
2LN-34	56.6%	11.2%
2LN-35	66.5%	6.6%

The table illustrates that clones from both the Sp2/0 and NS-1 fusions were able to produce antibodies that had a greater than 50% killing rate for cancerous cells and at the same time some of the clones were able to produce less than one percent killing of normal control fibroblasts.

Example 3

In this example antibodies were produced to several different breast tumor samples following the method of Example 2 in order to demonstrate the generality of producing customized antibodies. Biopsy specimens of breast tumors were obtained and stored at -70°C until used as noted in Example 1. Single cell suspensions were prepared for each specimen and fixed with -30°C, 70% ethanol, washed with PBS and reconstituted to an appropriate volume for injection. Female, 7-8 week old, A strain, H-2^d haplotype Balb/c mice (Charles River Canada,

1 St. Constant, QC, Can), were immunized with 2.5×10^5 - 1×10^6
2 cells and boosted every third week until a final pre-
3 fusion boost was performed three days prior to the
4 splenectomy. The hybridomas were prepared by fusing the
5 isolated splenocytes with Sp2/0 myeloma partners. The
6 supernatants from the fusions were tested for subcloning
7 of the hybridomas.

8
9 Hs574.T breast ductal carcinoma cells, A2058
10 melanoma cells, NCI-H460 human lung large cell carcinoma,
11 NCI-H661 human lung large cell carcinoma, CCD-112CoN human
12 colon fibroblasts, CCD-27sk human skin fibroblasts, MCF-
13 12A human mammary epithelial cells, Hs574.mg human breast
14 cells and other cell lines, were obtained from ATCC and
15 cultured according to enclosed instructions. Both cancer
16 and non-cancer cells were plated three to four days prior
17 to screening.

18 The hybridomas were cultured for ten to twelve days
19 after fusion and observed under the microscope. When 20 to
20 25% of the wells were over 80% confluent, the hybridoma
21 supernatants were screened in a cytotoxicity assay. The
22 hybridoma supernatants were divided into two 75 microliter
23 portions; one portion was added to a target cancer cell
24 plate and another to a non-cancer cell plate. Prior to
25 transfer of hybridoma supernatants, the cell plates were
26 washed three times with 100 microliters of PBS. The
27 supernatant from the anti-breast cancer hybridomas were
28 transferred to the Hs574.T and the Hs574.mg cells, whereas
29 the supernatant from the anti-lung cancer hybridoma were
30 transferred to the NCI-H460 and CCD-27SK cells. The

1 cancer cells were incubated with the hybridoma
2 supernatants for 18 hours at 37 degrees Celsius in an 8
3 percent CO₂ incubator.

4 The Live/Dead cytotoxicity assay was obtained from
5 Molecular Probes (Eugene,OR). The assays were performed
6 according to the manufacturer's instructions with the
7 changes outlined below. The plates with the cells were
8 washed once with 100 microliters of PBS at 37°C. 75 to 100
9 microliters of supernatant from the hybridoma microtitre
10 plates were transferred to the cell plates and incubated
11 in a 8% CO₂ incubator for 18-24 hours. Then, the wells that
12 served as the dead control cells were aspirated until
13 empty and 50 microliters of 70% ethanol was added. The
14 plate was then emptied by inverting and blotted dry. Room
15 temperature PBS was dispensed into each well from a
16 multichannel squeeze bottle, tapped three times, emptied
17 by inversion and then blotted dry. 50 microliters of the
18 fluorescent Live/Dead dye diluted in PBS was added to each
19 well and incubated at 37°C in a 5% CO₂ incubator for one
20 hour. The plates were read in a Perkin-Elmer HTS7000
21 fluorescence plate reader and the data was analyzed in
22 Microsoft Excel (Microsoft, Redmond, WA).

23 Four rounds of screening were conducted to
24 produce single clone hybridoma cultures. For two rounds of
25 screening the hybridoma supernatants were tested only
26 against the cancer cells. In the last round of screening
27 the supernatant was tested against a number of non-cancer

1 cells as well as the target cells indicated in Table 3.
2 The antibodies were isotyped using a commercial isotyping
3 kit (Roche, Indianapolis, IN).

4 A number of monoclonal antibodies were produced in
5 accordance with the method of the present invention.
6 These antibodies, whose characteristics are summarized in
7 Table 3, are identified as 4BD-1, 4BD-3, 4BD-6, 4BD-9,
8 4BD-13, 4BD-18, 4BD-20, 4BD-25, 4BD-37, 4BD-32, 4BD-26,
9 4BD-27, 4BD-28, 4BD-50, 6BD-1, 6BD-3, 6BD-5, 6BD-11, 6BD-
10 25, 7BD-7, 7BD-12-1, 7BD-12-2, 7BD-13, 7BD-14, 7BD-19,
11 7BD-21, 7BD-24, 7BD-29, 7BD-30, 7BD-31, 7BDI-17, 7BDI-58,
12 7BDI-60 and 7BDI-62. Each of the designated antibodies is
13 produced by a hybridoma cell line deposited with the
14 American Type Culture Collection at 10801 University
15 Boulevard, Manassas, Va. having an ATCC Accession Number
16 as follows:

17	<u>Antibody</u>	<u>ATCC Accession Number</u>
18	4BD-1	
19	4BD-3	
20	4BD-6	
21	4BD-9	
22	4BD-13	
23	4BD-18	
24	4BD-20	
25	4BD-25	
26	4BD-37	
27	4BD-32	

- 1 4BD-26
- 2 4BD-27
- 3 4BD-28
- 4 4BD-50
- 5 6BD-1
- 6 6BD-3
- 7 6BD-5
- 8 6BD-11
- 9 6BD-25
- 10 7BD-7
- 11 7BD-12-1
- 12 7BD-12-2
- 13 7BD-13
- 14 7BD-14
- 15 7BD-19
- 16 7BD-21
- 17 7BD-24
- 18 7BD-29
- 19 7BD-30
- 20 7BD-31
- 21 7BDI-17
- 22 7BDI-58
- 23 7BDI-60
- 24 7BDI-62

25 These antibodies are considered monoclonal after four
26 rounds of limiting dilution cloning. The panel of anti-
27 breast cancer antibodies killed 15-79% of the target cells
28 and <1-31% of the control cells. The majority of anti-

1 tumor antibodies were IgM type, suggesting they could be
2 directed against carbohydrate antigens on the surface of
3 tumor cells. There is a high therapeutic index since most
4 of the antibodies do not cause the normal cells to undergo
5 cell death.

6 These monoclonal antibodies are characterized for a
7 number of immunological and biochemical parameters. A
8 cell based enzyme linked immunosorbent assay (ELISA) was
9 established for measuring the binding of the antibodies
10 derived of each clones to different cell lines. Cells were
11 seeded and grown on 96-well tissue culture plates. The
12 plates were washed with 100 microliters of PBS. 100
13 microliters of cold 4 percent paraformaldehyde in PBS were
14 added to each well for ten minutes and then aspirated. The
15 plates were washed with PBS using a multichannel squeeze
16 bottle . The wells were emptied and 100 microliters of
17 blocking buffer (1 percent hydrocasein, 0.1 percent
18 geletin in 50mM Tris-HCl buffer, pH 9.3) was added to each
19 well for one hour at room temperature. The plates were
20 washed three times with a buffer (0.05 percent Tween 20 in
21 10 mM PBS) at room temperature and then stored at -30
22 degrees Celsius with 100 microliters of the buffer. Prior
23 to use the plates were thawed and the buffer was aspirated
24 from each well. 75 microliters of hybridoma supernatant
25 were added to each well and incubated for 60 minutes at
26 room temperature. After the plates were washed with PBS
27 using a multichannel squeeze bottle, 50 microliters of a

1 combination of peroxidase conjugated goat anti-mouse IgG
2 and peroxidase conjugated donkey anti-mouse IgM (Jackson
3 ImmunoResearch Lab, Inc., West Grove, PA.) was added and
4 incubated for 30 minutes at room temperature. After the
5 last wash, 50 microliters of orthophenylene diamine (OPD)
6 (Sigma, St. Louis, MO) was added to each well and the
7 optical density was read at 492 nm on the HTS7000 plate
8 reader after adding equal volume of 1 N sulfuric acid.
9 Different clones show different profiles in binding to
10 different cells (Table 3). This indicates that they may
11 target different cell surface antigen and further suggests
12 the variable distribution of these antigen on the surface
13 of cancer cells. Those binding only to cancer cells but
14 not to normal cells could identify certain tumor-
15 associated antigen.

16

17 Table 3. Anti-Breast Cancer Antibodies

Clones	Isotype	% Cell Death		Binding to cell lines				
		Hs574.T	Hs574.mg	Hs574.T	Hs574.mg	NCI-H460	CCD-27sk	A2058
6BD-1	μ, κ	38.2	5	0.8	0.5	0.6	0.3	ND*
6BD-3	μ, κ	79	12	0.35	0.25	0.24	0.14	ND
6BD-5	μ, κ	57.3	8	1.0	0.3	0.14	0.25	ND
6BD-11	μ, κ	52.3	11	0.15	0.1	0.17	0.1	ND
6BD-25	μ, κ	33.3	2	0.15	0.1	0.2	0.1	ND
4BD-26	μ, κ	27	1.8	0.5	ND	ND	<0.1	ND
4BD-27	μ, κ	19.6	<1	0.9	ND	ND	0.5	ND
4BD-28	μ, κ	26.4	<1	0.8	ND	ND	<0.1	ND
4BD-32	μ, κ	41.7	4	0.8	ND	ND	<0.1	ND
4BD-50	μ, κ	20	<1	0.8	ND	ND	<0.1	ND
4BD-1	μ, κ	23	31	0.6	ND	ND	<0.1	ND
4BD-3	μ, κ	29.7	8.2	1.7	ND	ND	0.1	ND
4BD-6	μ, κ	17	<1	0.9	ND	ND	<0.1	ND
4BD-9	μ, κ	15	<1	0.6	ND	ND	<0.1	ND
4BD-13	μ, κ	31	<1	1.2	ND	ND	<0.1	ND
4BD-18	μ, κ	23.3	2.4	0.7	ND	ND	0.12	ND
4BD-20	μ, κ	45	<1	0.95	ND	ND	<0.1	ND
4BD-25	μ, κ	26	14.16	1.8	ND	ND	0.1	ND
4BD-37	μ, κ	30	<1	0.8	ND	ND	<0.1	ND
7BD-7	μ, κ	24	3	0.8	0.3	1.4	0.26	ND
7BD-12-1	μ, κ	22	6	0.36	0.16	0.43	0.1	ND
7BD-12-2	μ, κ	31	2	0.2	0.2	0.2	0.2	0.2
7BD-13	μ, κ	29	12	0.1	0.15	0.2	0.1	0.2
7BD-14	μ, κ	32	13	0.4	0.4	0.6	0.3	0.5
7BD-19	μ, κ	20	4	1.3	0.4	0.43	0.2	ND
7BD-21	μ, κ	21	13	0.4	0.5	0.25	0.3	ND
7BD-24	μ, κ	32	15	0.3	0.1	0.14	0.15	ND
7BD-29	μ, κ	15	16	0.3	0.24	0.14	0.16	ND
7BD-30	μ, κ	23	13	0.34	0.24	0.2	0.16	ND
7BD-31	μ, κ	28	10	0.3	0.4	0.4	0.3	0.4

1	7BDI-17	μ, κ	23	<1	0.75	ND	ND	ND	ND
2	7BDI-58	$\gamma 1, \kappa$	17.5	<1	0.77	ND	ND	ND	ND
3	7BDI-60	$\gamma 1, \kappa$	15	<1	0.73	ND	ND	ND	ND
4	7BDI-62		15	5	0.55	ND	ND	ND	ND

5 *ND: not done.

6 **Example 4**

7 In this example customized anti-cancer antibodies are
8 produced to a lung cancer sample by first obtaining
9 samples of the patient's tumor preparing single cell
10 suspensions which are then fixed for injection into mice
11 as noted in Example 1. After the completion of the
12 immunization schedule the hybridomas are produced from the
13 splenocytes. The hybridomas are screened against a variety
14 of cancer cell lines and normal cells in standard
15 cytotoxicity assays. Those hybridomas that are reactive
16 against cancer cell lines but are not reactive against
17 normal non-transformed cells are selected for further
18 propagation. Clones that were considered positive were
19 ones that selectively killed the cancer cells but did not
20 kill the non-transformed cells.

21 The lung cancer cells were isolated and cell lines
22 were cultured as described in Example 1. Female, 7-8 week
23 old, A strain, H-2^d haplotype Balb/c mice (Charles River
24 Canada, St. Constant, QC, Can), were immunized with human
25 lung cancer cells. The lung cancer cell suspensions were
26 emulsified in an equal volume of Freund's complete
27 adjuvant (FCA) for the first immunization and then in

1 Freund's incomplete adjuvant (FIA) for subsequent
2 immunizations at 0, 21, 45 days. 5×10^5 cells were used to
3 immunize each mouse either through a subcutaneous or
4 intra-peritoneal route. Immunized mice were sacrificed 3-4
5 days after the final immunization with human lung cancer
6 cells at 148 days, given intra-peritoneally, in PBS at pH
7 7.4. The spleens were harvested and the splenocytes were
8 divided into two aliquots for fusion with Sp2/0 myeloma
9 partners using the methods outlined in Example 1.

10 The screening was carried out 10 days after the
11 fusion against NCI-H460 and/or NCI-H661 cells and CCD-27SK
12 fibroblasts. Each pair of plates were washed with 100
13 microliters of room temperature PBS and then aspirated to
14 near dryness. Then 75 microliters of hybridoma supernatant
15 was added per well on each of the two plates. The spent
16 Sp2/0 supernatant was added to the control wells at the
17 same volume and the plates were incubated for around 18
18 hours at 37 degrees Celsius at a 8%CO₂, 98% relative
19 humidity incubator. Then each pair of plates was removed
20 and in the positive control wells 50 microliters of 70%
21 ethanol was substituted for the media for 4 seconds. The
22 plates were then inverted and washed with room temperature
23 PBS once and dried. Then 50 microliters of fluorescent
24 live/dead dye diluted in PBS (Molecular Probes Live/Dead
25 Kit) was added for one hour and incubated at 37 degrees
26 Celsius. The plates were then read in a Perkin-Elmer
27 fluorescent plate reader and the data analyzed using

1 Microsoft Excel. The wells that were considered positive
2 were subcloned and the same screening process was repeated
3 6 days later and then 13 days later. The result of the
4 last screening is outlined in Table 4 below. Antibodies
5 were characterized for binding to different cell lines
6 with a cellular ELISA according to the methods of Example
7 3. A number of monoclonal antibodies were produced in
8 accordance with the method of the present invention.
9 These antibodies, whose characteristics are summarized in
10 Table 4, are identified as 5LAC2, 5LAC4, 5LAC20, and
11 5LAC23. Each of the designated antibodies is produced by
12 a hybridoma cell line deposited with the American Type
13 Culture Collection at 10801 University Boulevard,
14 Manassas, Va. having an ATCC Accession Number as follows:

15	<u>Antibody</u>	<u>ATCC Accession Number</u>

16 5LAC2

17 5LAC4

18 5LAC20

19 5LAC23.

20 Table 4. Anti-Lung Cancer Antibodies

Clones	Isotype	% Cell Death					Binding to cell lines				
		Hs574.T	NCH460	NCH4661	A2058	CCD-27sk	Hs574.T	Hs574.mg	NCH-4660	CCD-27sk	A2058
5LAC2	μ , κ	30	7	45.3	23	<1	0.2	0.2	0.26	0.2	0.2
5LAC4	μ , κ	21	11	20.5	23	3	0.7	0.9	1.7	0.8	0.9
5LAC20	μ , κ	23	7	66.4	24	3	0.5	0.2	0.6	0.2	0.2
		23	8	57.6	25	5	0.6	0.6	0.6	0.6	0.6

1 *ND: not done

2 The table illustrates that clones were able to
3 produce antibodies that had a greater than 7-67% killing
4 rate for cancerous cells and at the same time some of the
5 clones were able to produce less than five percent killing
6 of normal control fibroblasts.

7

8 **Example 5**

9 In this example customized anti-cancer antibodies are
10 produced to a patient's lung cancer cells, but cultured
11 cells were used in the antibody development process to
12 demonstrate the generality of the immunization process.
13 The samples were prepared into single cell suspensions and
14 fixed for injection into mice as noted in Example 1. After
15 the completion of three rounds of immunization with cells
16 derived directly from a patient's lung cancer, the mice
17 were immunized twice with a human lung large cell
18 carcinoma cell line (NCI-H460). Hybridomas were produced
19 from splenocytes and the supernatants were screened
20 against a variety of cancer cell lines and normal cells in
21 standard cytotoxicity assays. Those hybridomas that were
22 reactive against cancer cell lines but were not reactive
23 against normal non-transformed cells were selected for
24 further propagation. Clones that were considered positive
25 were ones that selectively killed the cancer cells but did
26 not kill the non-transformed cells. The antibodies are

1 characterized for a large number of biochemical parameters
2 and then humanized for therapeutic use.

3 The lung tumor cells isolated and cell lines were
4 cultured as described in Example 1. Balb/c mice, A strain
5 with H-2^d haplotype from Charles River Canada, St.
6 Constant, Quebec, Canada, female, 7-8 week old, were
7 immunized with the human lung cancer cells emulsified in
8 an equal volume of either Freund's complete adjuvant (FCA)
9 for the first immunization and then in Freund's incomplete
10 adjuvant (FIA) for subsequent immunizations at 0, 21, 45
11 days with 5×10^5 cells. The mice were immunized with fixed
12 NCI H460 cells, which were prepared from NCI H460 cells
13 grown in T-75 cell culture flask by scraping mono-layer
14 cells into cell suspensions at 105, 150 and 170 days.
15 Immunized mice were sacrificed 3-4 days after the final
16 immunization with NCI H460 cells, given intra-
17 peritoneally, in phosphate buffered saline buffer (PBS),
18 pH 7.4. The spleens were harvested and the splenocytes
19 were divided into two aliquots for fusion with Sp2/0
20 myeloma partners using the methods outlined in Example 1.

21 The screening was carried out 10 days after the
22 fusion against NCI H460 cells and CCD-27SK fibroblasts as
23 described in Example 4. Antibodies were characterized for
24 binding to different cell lines with a cellular ELISA
25 according to the methods of Example 3.

26

1 The wells that were considered positive were
2 subcloned and the same screening process was repeated 9
3 days and 18 days later. The results are outlined in Table
4 5 below. A number of monoclonal antibodies were produced
5 in accordance with the method of the present invention.
6 These antibodies, whose characteristics are summarized in
7 Table 5, are identified as H460-1, H460-4, H460-5, H460-
8 10, H460-14, H460-16-1, H460-16-2, H460-23 and H460-27.
9 Each of the designated antibodies is produced by a
10 hybridoma cell line deposited with the American Type
11 Culture Collection at 10801 University Boulevard,
12 Manassas, Va. having an ATCC Accession Number as follows:

13	<u>Antibody</u>	<u>ATCC Accession Number</u>
14	H460-1	
15	H460-4	
16	H460-5	
17	H460-10	
18	H460-14	
19	H460-16-1	
20	H460-16-2	
21	H460-23	
22	H460-27	

1 Table 5. Anti-Lung Cancer Antibodies

Clones	isotype	% Cell Death				Binding to cell lines				
		NCI-H460	Hs574.	A2058	CCD-	Hs574.	Hs574.	NCI-	CCD-	A2058
H460-1	$\gamma 1, \hat{e}$	16	30	23	<1	1.0	0.6	0.5	0.7	ND
H460-4		37	21	23	3	1.0	0.6	0.4	0.6	ND
H460-5	μ, κ	22.5	23	24	3	1.0	0.3	0.3	0.2	ND
H460-10	μ, κ	8	23	25	5	0.3	0.14	0.2	0.1	ND
H460-14	$\gamma 1, \hat{e}$	17	ND	ND	4	1.1	0.6	0.4	0.54	ND
H460-16-1	$\gamma 1, \hat{e}$	33	ND	ND	8	1.0	0.6	0.3	0.5	ND
H460-16-2	$\gamma 1, \hat{e}$	22	ND	ND	3	1.0	0.6	0.3	0.7	ND
H460-22-1	$\gamma 1, \hat{e}$	21	ND	ND	5	0.6	0.4	0.3	0.4	ND
H460-22-2	μ, κ	23	ND	ND	3	0.4	0.1	0.1	0.1	ND
H460-23	μ, κ	36	36	18	1	0.4	1.1	0.54	0.53	0.58
H460-27	μ, κ	33	31	16	8	0.3	0.4	0.4	0.3	0.4

16 *ND: not done

17 The table illustrates that clones were able to
 18 produce antibodies that had a greater than 15% killing
 19 rate for cancerous cells and at the same time some of the
 20 clones were able to produce less than eight percent
 21 killing of normal control fibroblasts.

22 The anti-cancer antibodies of the invention are
 23 useful for treating a patient with a cancerous disease
 24 when administered in admixture with a pharmaceutically
 25 acceptable adjuvant, for example normal saline, a lipid
 26 emulsion, albumen, phosphate buffered saline or the like
 27 and are administered in an amount effective to mediate
 28 treatment of said cancerous disease, for example with a

1 range of about 1 microgram per milliliter to about 1 gram
2 per milliliter.

3 The method for treating a patient suffering from a
4 cancerous disease may further include the use of
5 conjugated anti-cancer antibodies and would this include
6 conjugating patient specific anti-cancer antibodies with a
7 member selected from the group consisting of toxins,
8 enzymes, radioactive compounds, and hematogenous cells;
9 and administering these conjugated antibodies to the
10 patient; wherein said anti-cancer antibodies are
11 administered in admixture with a pharmaceutically
12 acceptable adjuvant, for example normal saline, a lipid
13 emulsion, albumen, phosphate buffered saline or the like
14 and are administered in an amount effective to mediate
15 treatment of said cancerous disease, for example with a
16 range of about 1 microgram per mil to about 1 gram per
17 mil. In a particular embodiment, the anti-cancer
18 antibodies useful in either of the above outlined methods
19 may be a humanized antibody.

20 The anti-cancer antibodies of the invention are
21 useful for treating a patient with a cancerous disease
22 when administered in admixture with a pharmaceutically
23 acceptable adjuvant, for example normal saline, a lipid
24 emulsion, albumen, phosphate buffered saline or the like
25 and are administered in an amount effective to mediate
26 treatment of said cancerous disease, for example with a

1 range of about 1 microgram per mil to about 1 gram per
2 mil.

3 The method for treating a patient suffering from a
4 cancerous disease may further include the use of
5 conjugated anti-cancer antibodies and would this include
6 conjugating patient specific anti-cancer antibodies with a
7 member selected from the group consisting of toxins,
8 enzymes, radioactive compounds, and hematogenous cells;
9 and
10 administering these conjugated antibodies to the patient;
11 wherein said anti-cancer antibodies are administered in
12 admixture with a pharmaceutically acceptable adjuvant, for
13 example normal saline, a lipid emulsion, albumen,
14 phosphate buffered saline or the like and are administered
15 in an amount effective to mediate treatment of said
16 cancerous disease, for example with a range of about 1
17 microgram per mil to about 1 gram per mil. In a
18 particular embodiment, the anti-cancer antibodies useful
19 in either of the above outlined methods may be a humanized
20 antibody.

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CLAIMS

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What is claimed is:

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Claim 1. A method for treating a patient suffering from a cancerous disease comprising:

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administering to said patient anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating a cancerous disease, said antibodies including a subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, said subset being essentially benign to non-cancerous cells;

wherein one or more antibodies or fragments thereof selected from said subset are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease;

said one or more antibodies or fragments thereof being selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a

1 H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a
2 H460-23 and a H460-27 monoclonal antibody or combinations
3 thereof.
4

5 Claim 2. The method for treating a patient suffering
6 from a cancerous disease in accordance with claim 1,
7 wherein said one or more antibodies or fragments thereof
8 selected from said subset are humanized.
9

10 Claim 3. The method for treating a patient suffering
11 from a cancerous disease in accordance with claim 1
12 comprising:

13 conjugating said subset of antibodies or fragments
14 thereof with a member selected from the group consisting
15 of toxins, enzymes, radioactive compounds, and
16 hematogenous cells; and

17 administering conjugated antibodies or fragments
18 thereof to said patient;

19 wherein said conjugated antibodies are placed in
20 admixture with a pharmaceutically acceptable adjuvant and
21 are administered in an amount effective to mediate
22 treatment of said cancerous disease.
23

24 Claim 4. The method of claim 3, wherein said one or
25 more antibodies or fragments thereof selected from said
26 subset are humanized.

1 Claim 5. The method for treating a patient suffering
2 from a cancerous disease in accordance with claim 1
3 wherein:

4 the cytotoxicity of said antibodies or fragments
5 thereof is mediated through antibody dependent cellular
6 toxicity.

7

8 Claim 6. The method for treating a patient suffering
9 from a cancerous disease in accordance with claim 1
10 wherein:

11 the cytotoxicity of said antibodies or fragments
12 thereof is mediated through complement dependent cellular
13 toxicity.

14

15 Claim 7. The method for treating a patient suffering
16 from a cancerous disease in accordance with claim 1
17 wherein:

18 the cytotoxicity of said antibodies or fragments
19 thereof is mediated through catalyzing of the hydrolysis
20 of cellular chemical bonds.

21

22 Claim 8. The method for treating a patient suffering
23 from a cancerous disease in accordance with claim 1
24 wherein:

25 the cytotoxicity of said antibodies or fragments
26 thereof is mediated through producing an immune response
27 against putative cancer antigens residing on tumor cells.

1 Claim 9. The method for treating a patient suffering
2 from a cancerous disease in accordance with claim 1
3 wherein:

4 the cytotoxicity of said antibodies or fragments
5 thereof is mediated through targeting of cell membrane
6 proteins to interfere with their function.

7

8 Claim 10. The method for treating a patient suffering
9 from a cancerous disease in accordance with claim 1
10 wherein:

11 the cytotoxicity of said antibodies or fragments
12 thereof is mediated through production of a conformational
13 change in a cellular protein effective to produce a signal
14 to initiate cell-killing.

15

16 Claim 11. The method for treating a patient suffering
17 from a cancerous disease in accordance with claim 1
18 wherein:

19 said method of production utilizes a tissue sample
20 containing cancerous and non-cancerous cells obtained from
21 a particular individual.

22

23 Claim 12. A method for treating a patient suffering
24 from a cancerous disease comprising:

25 administering to said patient anti-cancer antibodies
26 or fragments thereof produced in accordance with a method
27 for the production of individually customized anti-cancer
28 antibodies which are useful in treating a cancerous

1 disease, said antibodies including a subset of antibodies
2 or fragments thereof characterized as being cytotoxic
3 against cells of a cancerous tissue, said subset being
4 essentially benign to non-cancerous cells;

5 wherein one or more antibodies or fragments thereof
6 selected from said subset are placed in admixture with a
7 pharmaceutically acceptable adjuvant and are administered
8 in an amount effective to mediate treatment of said
9 cancerous disease;

10 said one or more antibodies or fragments thereof
11 produced by a hybridoma cell line having an ATCC Accession
12 Number selected from the group consisting of (to be
13 provided before publication) or combinations thereof.

14

15 Claim 13. The method for treating a patient suffering
16 from a cancerous disease in accordance with claim 12,
17 wherein said one or more antibodies or fragments thereof
18 selected from said subset are humanized.

19

20 Claim 14. The method for treating a patient suffering
21 from a cancerous disease in accordance with claim 12
22 comprising:

23 conjugating said subset of antibodies or fragments
24 thereof with a member selected from the group consisting
25 of toxins, enzymes, radioactive compounds, and
26 hematogenous cells; and

27 administering conjugated antibodies or fragments
28 thereof to said patient;

1 wherein said conjugated antibodies are placed in
2 admixture with a pharmaceutically acceptable adjuvant and
3 are administered in an amount effective to mediate
4 treatment of said cancerous disease.

5
6 Claim 15. The method of claim 14, wherein said one or
7 more antibodies or fragments thereof selected from said
8 subset are humanized.

9
10 Claim 16. The method for treating a patient suffering
11 from a cancerous disease in accordance with claim 12
12 wherein:

13 the cytotoxicity of said antibodies or fragments
14 thereof is mediated through antibody dependent cellular
15 toxicity.

16
17 Claim 17. The method for treating a patient suffering
18 from a cancerous disease in accordance with claim 12
19 wherein:

20 the cytotoxicity of said antibodies or fragments
21 thereof is mediated through complement dependent cellular
22 toxicity.

23
24 Claim 18. The method for treating a patient suffering
25 from a cancerous disease in accordance with claim 12
26 wherein:

27

1 the cytotoxicity of said antibodies or fragments
2 thereof is mediated through catalyzing of the hydrolysis
3 of cellular chemical bonds.

4

5 Claim 19. The method for treating a patient suffering
6 from a cancerous disease in accordance with claim 12
7 wherein:

8 the cytotoxicity of said antibodies or fragments
9 thereof is mediated through producing an immune response
10 against putative cancer antigens residing on tumor cells.

11

12 Claim 20. The method for treating a patient suffering
13 from a cancerous disease in accordance with claim 12
14 wherein:

15 the cytotoxicity of said antibodies or fragments
16 thereof is mediated through targeting of cell membrane
17 proteins to interfere with their function.

18

19 Claim 21. The method for treating a patient suffering
20 from a cancerous disease in accordance with claim 12
21 wherein:

22 the cytotoxicity of said antibodies or fragments
23 thereof is mediated through production of a conformational
24 change in a cellular protein effective to produce a signal
25 to initiate cell-killing.

26

27

1 Claim 22. The method for treating a patient suffering
2 from a cancerous disease in accordance with claim 12
3 wherein:
4 said method of production utilizes a tissue sample
5 containing cancerous and non-cancerous cells obtained from
6 a particular individual.

7
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10 Claim 23. Anti-cancer antibodies or fragments thereof
11 selected from the group consisting of a 1LN-8, 4BD-1, a
12 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a
13 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37,
14 a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a
15 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-
16 19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a
17 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a
18 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a H460-5, a
19 H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-23
20 and a H460-27 monoclonal antibody or combinations thereof.

21
22
23 Claim 24. Anti-cancer antibodies or fragments thereof
24 produced by a hybridoma cell line having an ATCC Accession
25 Number selected from the group
26 consisting of (to be provided before publication).

27
28

1 Claim 25. The use of a composition for treating a
2 patient suffering from a cancerous disease by
3 administration of an effective amount of said composition
4 to a patient to mediate treatment of said cancerous
5 disease, wherein said composition comprises one or more
6 antibodies or fragments thereof selected from a subset of
7 said antibodies or fragments in admixture with a
8 pharmaceutically acceptable adjuvant, said anti-cancer
9 antibodies or fragments thereof produced in accordance
10 with a method for the production of individually
11 customized anti-cancer antibodies which are useful in
12 treating cancerous disease, said subset of antibodies or
13 fragments thereof characterized as being cytotoxic against
14 cells of a cancerous tissue, as being essentially benign
15 to non-cancerous cells and being selected from the group
16 consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a
17 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27,
18 a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3,
19 a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-
20 12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a
21 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-
22 60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a
23 H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-
24 16-1, a H460-16-2, a H460-23 and a H460-27 monoclonal
25 antibody or combinations thereof.

26

27

1 Claim 26. The use of a composition for treating a
2 patient suffering from a cancerous disease by
3 administration of an effective amount of the composition
4 to the patient to mediate treatment of said cancerous
5 disease, wherein said composition comprises one or more
6 antibodies or fragments thereof from a subset of
7 antibodies or fragments thereof characterized as being
8 cytotoxic against cells of a cancerous tissue and
9 essentially benign to non-cancerous cells placed in
10 admixture with a pharmaceutically acceptable adjuvant,
11 said one or more antibodies or fragments thereof produced
12 by a hybridoma cell line having an ATCC Accession Number
13 selected from the group consisting of (to be provided
14 before publication) or combinations thereof.

Received by the International Bureau on 28 April 2003 (28.04.2003)
original claims 1-26 replaced by amended claims 1-25 (04 pages).

1

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What is claimed is:

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Claim 1. A method for treating a patient suffering
from a cancerous disease comprising:

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administering to said patient anti-cancer antibodies
or fragments thereof produced in accordance with a method
for the production of individually customized anti-cancer
antibodies which are useful in treating a cancerous
disease, said antibodies including a subset of antibodies
or fragments thereof characterized as being cytotoxic
against cells of a cancerous tissue, said subset being
essentially benign to non-cancerous cells;

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wherein one or more antibodies or fragments thereof
selected from said subset are placed in admixture with a
pharmaceutically acceptable adjuvant and are administered
in an amount effective to mediate treatment of said
cancerous disease;

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said one or more antibodies or fragments thereof
being selected from the group consisting of a 1LN-8, 4BD-
1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-
20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a
4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a
6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-
14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a
7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a
5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a

1 H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2,
2 a H460-22-1, a H460-23 and a H460-27 monoclonal antibody or
3 combinations thereof.
4

5 Claim 2. The method for treating a patient suffering
6 from a cancerous disease in accordance with claim 1,
7 wherein said one or more antibodies or fragments thereof
8 selected from said subset are humanized.
9

10 Claim 3. The method for treating a patient suffering
11 from a cancerous disease in accordance with claim 1
12 comprising:

13 conjugating said subset of antibodies or fragments
14 thereof with a member selected from the group consisting
15 of toxins, enzymes, radioactive compounds, and
16 hematogenous cells; and

17 administering conjugated antibodies or fragments
18 thereof to said patient;

19 wherein said conjugated antibodies are placed in
20 admixture with a pharmaceutically acceptable adjuvant and
21 are administered in an amount effective to mediate
22 treatment of said cancerous disease.
23

24 Claim 4. The method of claim 3, wherein said one or
25 more antibodies or fragments thereof selected from said
26 subset are humanized.

1 Claim 22. The method for treating a patient suffering
2 from a cancerous disease in accordance with claim 12
3 wherein:

4 said method of production utilizes a tissue sample
5 containing cancerous and non-cancerous cells obtained from
6 a particular individual.
7
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9

10 Claim 23. Anti-cancer antibodies or fragments thereof
11 selected from the group consisting of a 1LN-8, 4BD-1, a
12 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a
13 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37,
14 a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a
15 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-
16 19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a
17 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a
18 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a H460-5, a
19 H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-22-1,
20 a H460-23 and a H460-27 monoclonal antibody or combinations
21 thereof.
22

23 Claim 24. Anti-cancer antibodies or fragments thereof
24 produced by a hybridoma cell line having an ATCC Accession
25 Number selected from the group
26 consisting of (to be provided before publication).
27
28

1 Claim 25. The use of a composition for treating a
2 patient suffering from a cancerous disease by
3 administration of an effective amount of said composition
4 to a patient to mediate treatment of said cancerous
5 disease, wherein said composition comprises one or more
6 antibodies or fragments thereof selected from a subset of
7 said antibodies or fragments in admixture with a
8 pharmaceutically acceptable adjuvant, said anti-cancer
9 antibodies or fragments thereof produced in accordance
10 with a method for the production of individually
11 customized anti-cancer antibodies which are useful in
12 treating cancerous disease, said subset of antibodies or
13 fragments thereof characterized as being cytotoxic against
14 cells of a cancerous tissue, as being essentially benign
15 to non-cancerous cells and being selected from the group
16 consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a
17 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27,
18 a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3,
19 a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-
20 12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a
21 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-
22 60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a
23 H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-
24 16-1, a H460-16-2, a H460-22-1, a H460-23 and a H460-27
25 monoclonal antibody or combinations thereof.

26

27

STATEMENT UNDER ARTICLE 19 (1)

The amendments include:

(1) amendments to claim 1 to correct editorial errors.

(2) amendments to claims 1, 23 and 25 to insert reference to "a H460-22-1," in the three claims, support for this appearing in Table 5 on page 37, line 13.

To provide consistency with the above, a new disclosure page 36 is included with the amended claim pages.

Finally, attached hereto are copies of submissions and acknowledgement of the submission of Deposited Material and certain ATCC Numbers in relation to a related and counterpart U.S. application number 09/727,361 as provided to us by the instructing U.S. Attorney. This material also makes reference to H460-22-1.

The cell line referred to in the ATCC material is found in the disclosure at the noted pages hereinbelow:

<u>Cell Line</u>	<u>Reference Pages</u>
1LN-8	22 (23)
5LAC20	33 (33)
3BD-26	19 (20)
3BD-8	19 (20)
7BD-14	27 (30)
3BD-27	19 (20)
H460-27	36 (37)
H460-23	36 (37)
H460-16-2	36 (37)
H460-22-1	36 (37) (amended as above)
7BDI-60	27 (31)

The first reference page number being reference to the page where the cell line is listed, whereas the second bracketed number is the page of the table in which the cell line appears.

Appropriate amendments to the disclosure to add the ATCC numbers will be effected in due course.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE


IN RE APPLICANT : Young et al.
 INVENTION : **Individualized Anti-Cancer Antibodies**
 SERIAL NUMBER : 09/727,361
 FILING DATE : November 29, 2000
 EXAMINER : Susan Ungar
 GROUP ART UNIT : 1642
 OUR FILE NO. : 2056.009

To: The Commissioner of Patents and Trademarks
 Washington, D.C. 20231

CORROBORATION FOR DEPOSITED MATERIALS

I, Ferris H. Lander, a person in a position to corroborate the identity of the hybridoma cell lines 1LN-8 (shown in the table on page 30), 3BD-8 (shown in the table on page 26), 3BD-26 (shown in the table on page 26), 3BD-27 (shown in the table on page 26), H460-27 (shown in the table on page 45), H460-23 (shown in the table on page 45), 7BD-14 (shown in the table on page 35) and 5LAC20 (shown in the table on page 41) which were deposited, in accordance with the Budapest Treaty, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209 on November 21, 2000 under Accession Numbers PTA-2693, PTA-2696, PTA-2695, PTA-2698, PTA-2699, PTA-2700, PTA-2697 and PTA 2694 respectively. And additionally, the hybridoma cell lines H460-16-2 (shown in the table on page 45) and 7BDI-60 (shown in the table on page 39) which were deposited on September 4, 2002 under Accession Numbers PTA-4621 and PTA-4623 respectively, do hereby state that the deposited hybridoma are the same hybridoma cell lines disclosed and claimed in the above-referenced patent application.

Respectfully submitted,


 Ferris H. Lander
 Registration # 43,377

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ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Arius Research Inc.
Attn: Lisa Cechetto
6299 Airport Road
Mississauga, ON
L4V 1N3
Canada

Deposited on Behalf of: Arius Research Inc.

Identification Reference by Depositor:

Mouse hybridoma cell line: 1LN8
Mouse hybridoma cell line: 5LAC20
Mouse hybridoma cell line: 3BD26
Mouse hybridoma cell line: 3BD8
Mouse hybridoma cell line: 7BD14
Mouse hybridoma cell line: 3BD27
Mouse hybridoma cell line: H460-27
Mouse hybridoma cell line: H460-23

Patent Deposit Designation

PTA-2693
PTA-2694
PTA-2695
PTA-2696
PTA-2697
PTA-2698
PTA-2699
PTA-2700

The deposits were accompanied by: a scientific description, a proposed taxonomic description indicated above. The deposits were received November 21, 2000 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

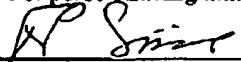
If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested November 30, 2000. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:



Frank Simone, Director, Patent Depository

Date: December 21, 2000

cc: Mr. Ferris Lander (Ref: Docket or Case No.: 09/415,278)

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

**BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE**

INTERNATIONAL FORM

**RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.**

To: (Name and Address of Depositor or Attorney)

Arius Research Inc.
Attn: Jean de Sousa-Hitzler
55 York Street 16th Floor
Toronto, Ontario
Canada M5J 1R7

Deposited on Behalf of: Arius Research Inc.

Identification Reference by Depositor:

Patent Deposit Designation

Mouse Hybridoma: H460-16-2
Mouse Hybridoma: H460-22-1
Mouse Hybridoma: 7BD1-60

PTA-4621
PTA-4622
PTA-4623

The deposits were accompanied by: a scientific description, a proposed taxonomic description indicated above. The deposits were received September 4, 2002 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested September 6, 2002. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Marie Harris
Marie Harris, Patent Specialist, ATCC Patent Depository

Date: October 9, 2002

cc: Mr. Ferris Lander
(Ref: Docket or Case No.: 2056.009 & US Serial No. 09/727361)

INTERNATIONAL SEARCH REPORT

tional Application No

PCT/CA 01/01838

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61K47/48 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2001/009665 A1 (YOUNG DAVID S F ET AL) 26 July 2001 (2001-07-26) the whole document ---	1-26
X	US 2001/003777 A1 (YOUNG DAVID S F ET AL) 14 June 2001 (2001-06-14) the whole document ---	1-26
X	US 6 180 357 B1 (YOUNG DAVID S F ET AL) 30 January 2001 (2001-01-30) the whole document --- -/--	1-26

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

10 January 2003

Date of mailing of the international search report

16/01/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

COVONE-VAN HEES, M

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/CA 01/01838

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 20401 A (UNIV BOSTON) 3 August 1995 (1995-08-03) page 17, line 16-27 page 29, line 4-20 example 13 claims 30,31,38-40 -----	1-6, 11-17, 22-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 01/01838

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-22, 25, 26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

onal Application No

PCT/CA 01/01838

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2001009665 A1	26-07-2001	US 6180357 B1	30-01-2001
		US 2002041877 A1	11-04-2002
		US 2001003777 A1	14-06-2001
US 2001003777 A1	14-06-2001	US 6180357 B1	30-01-2001
		US 2002041877 A1	11-04-2002
		US 2001009665 A1	26-07-2001
US 6180357 B1	30-01-2001	US 2002041877 A1	11-04-2002
		US 2001009665 A1	26-07-2001
		US 2001003777 A1	14-06-2001
WO 9520401 A	03-08-1995	AU 1736495 A	15-08-1995
		EP 1231268 A2	14-08-2002
		EP 0744958 A1	04-12-1996
		US 6335163 B1	01-01-2002
		WO 9520401 A1	03-08-1995
		US 5789208 A	04-08-1998
		US 2001049107 A1	06-12-2001